The urine urease activity as a lab method to diagnose kidney stone formation in children with urolithiasis

Assel Sagymbayeva (✉ sagymbaeva.assel@gmail.com)  
Kazakh National Medical University

Natalya Merkusheva

Minira Bulegenova  
Scientific center of pediatrics and pediatric surgery, Kazakhstan

Bakitzhan Abekenov  
Scientific center of pediatrics and pediatric surgery, Kazakhstan

Anar Musabalina  
Scientific center of pediatrics and pediatric surgery, Kazakhstan

Abay Kussainov  
Scientific center of pediatrics and pediatric surgery, Kazakhstan

Research Article

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Abstract

Objective: To study the clinical efficiency of the urine Urease Activity (UA) test to diagnose kidney stones formation in children with urolithiasis. The prospective observational study was conducted at the Scientific Centre of Pediatrics and Pediatric Surgery (Almaty, Kazakhstan). This study included 80 patients: 40 patients with urolithiasis and 40 conditionally healthy children. The urine UA, standard urinalysis, and special methods of urine examination have been carried out for all patients. The clinical efficiency of the urine UA test was determined after comparison it with other lab tests and severity of urolithiasis among the different subgroups. The urine UA was correlated with the standard urinalysis. Patients with the high UA, between 110 and 400 mmol/l, also had the high numbers of WBC 187 [32-292], RBC 17 [3-34], and bacteria >10^5. These patients had most severe symptoms of urolithiasis including an inflammation. At the same time, low UA (0-50 mmol/l) were recorded for the patients with the mild symptoms. High level of UA was observed only in urine samples with phosphate and ammonium biurate crystals. The main bacterial pathogens in these cases were Klebsiella pneumonia and Pseudomonas aeruginosa. The values of UA were determined for the different types of crystals and uropathogens as well.

Conclusion: The urine UA is the clinically efficient test for the diagnosis of kidney stone formation and reflects the presence of active urease-forming bacteria in urine, that contributes to the formation of the infected stones.

What Is Known

• In most cases urine of patients with urolithiasis is infected with various bacteria, which produce the enzyme urease, that plays a leading role in the formation of the infected stones.

What Is New:

• The urine Urease Activity test is a valuable tool to diagnose the formation of infected stones in children with urolithiasis.

Introduction

Urolithiasis has a high recurrent rate because it is a multifactorial disease [1-4]. In fact, the lack of preventive measures for 5 years leads to recurrence in almost 50% of patients with urolithiasis. In more than 60% of all recurrences occur as early as 3 years after removal of the primary stone [6-8].

In most cases urine of patients with urolithiasis is infected with various bacteria [10, 15-19], most common are as follows: E. coli, Proteus mirabilis, Staphylococcus aureus, Pseudomonas aeruginosa [11]. Bacteria produce the enzyme Urease, that plays a leading role in the formation of the infected stones. This is one of the main mechanisms of crystal formation in urine and consequently calculus formation in
kidney [15]. Formerly the express test of urine UA was only performed in adult patients with urolithiasis [12-14], and was never used for the pediatric patients. We were studying the clinical efficiency of the urine UA test in children with urolithiasis.

Practically, the treatment of urolithiasis is usually limited to removal of calculus, and not enough attention has been given to the monitoring of kidney stone formation. One of the reasons for this, in our opinion, is the lack of the sufficient lab tests indicating the presence of crystal formation in urine, and consequently kidney stone formation. Despite of several proposed tests for this purpose [5,9], the study continues in this direction.

**Methods**

The prospective observational study was conducted in the Scientific Centre of Pediatrics and Pediatric Surgery, Almaty, Kazakhstan from September 2021 to July 2022. 40 children with diagnosis obstructing stone of the upper urinary tract (main group) and 40 conditionally healthy children (control group) were included into the study.

All patients with a verified clinical diagnosis of an obstructing stone of the upper urinary tract; completed instrumental investigations: abdominal X-ray, abdominal and urinary tract ultrasound were included in the main group. Some patients with urolithiasis that not required surgical treatment; patients with urinary tract abnormalities; patients who have refused to participate in the study; and didn't come to the follow-up examination; if urine of patients was collected from a urethral catheter or nephro-/pyelostoma were excluded from this study.

The control group consisted of healthy children who had come for the standard checkup to confirm that any problems from the urinary tract and kidneys were absent.

These two groups were compared by sex, age, and body mass index (BMI).

All children in both groups collected the morning urine to conduct the standard urinalysis and other special lab tests. The indicators such as pH, WBC, RBC, bacteria, crystals were obtained from the standard urinalysis using the strip-test Uriscan (Germany) for pH and the microscope for the rest of indicators. The bacteriological examination of urine was carried out by calculation of the number of colonies forming on the 5% blood agar or Cled agar divided by sectors [16].

The special urine tests included the determination of urine UA, pH stability, induced urine crystallization, urine Ability to Crystallize (AC) according to the developed techniques [12-14].

UA was compared with the other tests’ parameters to discover its efficiency. For this purpose, 40 children with urolithiasis were divided into 3 subgroups: the 1st subgroup (22 children) consisted of the patients whose UA was within the reference numbers; the 2nd subgroup included patients with a small increase of UA (7 children); and the 3rd subgroup contained of patients with very high numbers of UA (11 children).


**Statistical analysis**

Statistical analysis was performed using StatTech v. 2.8.8 (Developer - StatTech LLC, Kazan). Quantitative variables were assessed for compliance with the normal distribution using the Shapiro-Wilk criterion. Quantitative variables following a normal distribution were estimated using arithmetic mean (M) and standard deviation (SD), 95% confidence interval (95% CI). Quantitative variables following non-normal distribution were described using median (Me) and lower and upper quartiles (Q1 – Q3). Categorical data were described with absolute values and percentages. The three subgroups were compared for a quantitative measure that differed from the normal distribution using the Kruskal-Wallis test. Percentage comparisons in multifield matrix analyses were performed using Pearson’s chi-square test. The direction and closeness of the correlation between two quantitative variables were assessed using the Spearman rank correlation coefficient (for non-normal distributions). A predictive model describing the dependence of a quantitative variable on factors was developed using the linear regression method.

**Results**

The main and control groups of patients did not differ significantly in age, gender, and BMI. The patients’ age ranged from 3 months to 17 years (mean age 7±5 years) in the main group, the mean age in the control group was 8±4 years. The age category of patients with urolithiasis was dominated by children from 3 months to 5 years. There were 24 (60%) boys, 16 (40%) girls in the main group, and 20 (50%) boys, 20 (50%) girls in the control group.

In patients with urolithiasis the average size of the obstructing stone was 20 ± 3mm, and the Hounsfield stone density was 2050 [1800 - 2625]. The left kidney was affected in 23 (57.5%) patients, and the right kidney in the remaining 17 (43.5%) patients.

UA was significantly increased (< 0.001) in the main group (N=40) compared with the control group (N=40); 57 [50-200] mmol/l, median (IQR), and 0 [0-10] mmol/l respectively.

UA of urine was compared with other urine indicators detecting the process of inflammation in the kidney and urinary tract, and the process of crystal formation in urine. The obtained data was included in Table 1.

**Table 1** Comparative analysis of urine UA and other urine indicators
<table>
<thead>
<tr>
<th>Standard and other indicators</th>
<th>Children with urolithiasis</th>
<th>Conditionally healthy children</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=40</td>
<td>N=40</td>
<td></td>
</tr>
<tr>
<td>Low UA 0-50 mmol/L</td>
<td>Medium UA 60-100 mmol/L</td>
<td>High UA 110-400 mmol/L</td>
<td>UA 0-10 mmol/L</td>
</tr>
<tr>
<td>Number of patients N=22</td>
<td>Number of patients N=7</td>
<td>Number of patients N=11</td>
<td></td>
</tr>
<tr>
<td>pH, Median [Q – Q ]</td>
<td></td>
<td></td>
<td>0.004*</td>
</tr>
<tr>
<td>6.00 [6.00-6.00]</td>
<td>4.00 [4.00-6.00]</td>
<td>6.00 [6.00-7.00]</td>
<td>6.00 [5.50-6.50]</td>
</tr>
<tr>
<td>pH after 48 hours, Median [Q – Q ]</td>
<td></td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>6.00 [6.00-6.00]</td>
<td>4.00 [4.00-6.00]</td>
<td>8.00 [6.00-8.00]</td>
<td>6.00 [6.00-6.00]</td>
</tr>
<tr>
<td>White blood cells / WBC per mL, Median [Q – Q ]</td>
<td></td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>Red blood cells /RBC per mL, Median [Q – Q ]</td>
<td></td>
<td></td>
<td>0.191</td>
</tr>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>0</td>
<td>10^5</td>
<td>&gt;10^5</td>
<td>0</td>
</tr>
<tr>
<td>Presence of crystals in the urinalysis (number of cases)</td>
<td></td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>0</td>
<td>7</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Presence of crystals in urine after 48 hours (number of cases)</td>
<td></td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>22</td>
<td>7</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Urine Ability to Crystallize (AC) mmol/L, Median [Q – Q ]</td>
<td></td>
<td></td>
<td>0.001*</td>
</tr>
<tr>
<td>50 [48-50]</td>
<td>99 [88-100]</td>
<td>357 [208-400]</td>
<td>0 [0-10]</td>
</tr>
</tbody>
</table>

* – differences are statistically significant (p < 0.05)

P value was calculated by chi-square test or Kruskal-Wallis test.
In the 1st urolithiasis patients’ subgroup was a correlation between UA and pH, bacteria, crystals in comparison with healthy children, at the same time, a significant increase in urinary WBC and RBC was observed. The health condition of these patients was in better stage in comparison with the 3rd subgroup; they didn't have any severe symptoms of the urinary tract inflammation.

The special urine tests were conducted to detect the process of stone formation in kidney. This technique included urine pH stability, after 48 hours of thermostatting. In the presence of uropathogen, would be an increase in urine pH, that contributes to the formation of phosphate, carbonate, oxalate crystals in urine [12]. In patients of the 1st subgroup pH stability was within the normal range, as well as in healthy children, and it did not increase. Thus, urine UA was correlated with the urine pH stability test.

Crystalluria is an important indicator, but standard urinalysis does not always show the presence of crystals, so we used the induced crystallization test [14]. Healthy children had no crystals in their urine, whereas all 22 patients with urolithiasis had crystals grown after 48 hours. Thus, this test was informative for the diagnosis of crystal formation in urine. It was no direct correlation between the urine UA and induced crystal formation in this subgroup. This fact clarified that not only urease-forming bacteria were involved in crystal formation, but there were also other mechanisms.

Urine ability to crystallize (AC) indicates the presence of different chemical substances in the urine that can react with calcium ions. They can be both inorganic like phosphates, carbonates, oxalates and organic like proteins [12,13].

In healthy children AC was 0, whereas in 1st subgroup it increased up to 50 mmol/l and correlated with the urine UA.

In the 2nd subgroup higher UA correlated with the higher numbers of WBC, bacteria, crystals. The chemical composition of the urine crystals revealed the formation of Uric acid in the urine of most patients due to acidic pH 4. This urine had some urease producing bacteria, but its quantity was not enough to raise pH after 48 hours and induce the crystal formation. AC was significantly higher not only in comparison with healthy children, but also with the 1st group of patients. This revealed the other mechanism of crystal formation beside the urease enzyme activity.

All patients in this subgroup had abdominal or flank pain associated with the renal colic, the symptoms were similar or very close to those in patients in the 1st subgroup.

All patients in the 3rd subgroup had higher numbers of UA, WBC, RBC, bacteria and crystalluria in comparison not only with the healthy children, but also with the patients of first two subgroups; the urine pH increased up to 8 after 48 hours. All these tests confirmed the presence of urease-forming uropathogens in urine and detected an inflammatory process in kidney. The induced crystalluria was observed in all 11 patients, and the chemical composition of crystals shown mostly phosphates in various modifications. AC values were the highest in this subgroup as well. So, the main mechanism of
crystal formation in this subgroup was the urease-forming bacteria that contributed to the formation of the infected stones.

Patients from this subgroup had the severe symptoms of urolithiasis, including an acute inflammatory process, according to standard blood and urine tests, signs of urinary obstruction, persistent increase in body temperature to $38^\circ C$ for three days or more, decreased diuresis. Young children usually had vomiting as well. Some infants had the intrauterine infections and weight loss. The urine of most patients had unusual smell and abnormal color.

We noticed the direct connection between UA and AC. This fact had a logical explanation, the increase in the number of urease-forming bacteria in urine would theoretically lead to the increase in the urine's ability to crystallize. We statistically calculated this correlation and presented the results in Table 2.

**Table 2** Correlation between UA and AC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation characteristics</th>
<th>$\rho$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine Urease Activity – Urine's Ability to Crystallize</td>
<td>The closeness of the relationship on the Cheddock scale</td>
<td>0.997</td>
<td>$&lt; 0.001^*$</td>
</tr>
</tbody>
</table>

* – differences are statistically significant ($p < 0.05$)

The observed dependence of the urine AC on the urine UA is described by the equation of paired linear regression (Figure 1): $Y_{UA} = 1.11 \times X_{UA} - 2.119$

With an increase in UA by 1 mmol/L, an increase AC by 1.11 mmol/L should be expected. The resulting model explains 97.8% of the observed dispersion of the AC.

Statistically significant difference ($p < 0.001$) was found when comparing urine UA and type of crystalluria (Figure 2). High numbers of UA such as 324 mmol/l in 5 patients (12.5%) were accompanied by phosphate crystal formation; urates associated with UA 100 mmol/l; oxalates linked to 58 mmol/l; mixed oxalate-urate type of crystals observed at UA 50 mmol/l (Figure 2).

The crystal formation speed is also very important, because the process can be described as high, medium or low. High speed corresponded to 180-200 crystals and above, medium speed to 50-80-120 crystals, and low speed to less than 40 crystals in the field of view of the microscope. We performed a statistical analysis of the dependence of crystal formation rate on UA (Figure 3).

A high speed of crystal formation was observed in 10 (25%) patients with urolithiasis, middle in 6 (15%) and a low speed in 24 (60%) patients. For example, high UA (306 mmol/l) was accompanied by a high speed of crystal formation (200 crystals in 48hrs.).
Urine UA has been analyzed in association with the characteristics of the spectrum of urinary tract infections (Figure 4).

In our study we have found that very high UA was observed in the presence of Klebsiella pneumonia; medium UA occurred with Pseudomonas aeruginosa and with a combination of two or more bacteria; lower UA was in urine with Proteus mirabilis (62 mmol/l) and E. Coli (54 mmol/l). A similar low UA (49 mmol/l) was observed in urine with Enterococcus faecalis and Staphylococcus haemolyticus. Our data complied with other informational sources [17-19].

**Discussion**

UA of urine in urolithiasis patients was significantly higher compared with healthy individuals 57 [50-200] mmol/l and 0 [0-10] mmol/l respectively. It correlated with the standard indicators of urinalysis as WBC, RBC, bacteriuria and crystalluria. The high numbers of UA between 110 and 400 mmol/l were accompanied by the high numbers of WBC 187 [32-292], RBC 17 [3-34] and bacteria >10^5 in urine.

Patients with high numbers of UA in urine had the severe symptoms of urolithiasis, including an acute inflammatory process, according to standard blood and urine tests, signs of urinary obstruction, persistent increase in body temperature to 38°C for three days or more, decreased diuresis. Young children usually had vomiting as well. Some infants had the intrauterine infections and weight loss. The urine of most patients had unusual smell and abnormal color.

At the same time, low numbers of UA from 0 to 50mmol/l were recorded for the patients with low levels of inflammation: WBC 22 [16-24], RBC 5 [2-11], no bacteria and crystals in urine. All patients mentioned above had abdominal or flank pain associated with the renal colic, but they didn't have any severe symptoms of the urinary tract inflammation.

We have found the clear connection between the presence of uropathogen and the UA of urine, confirmed by the standard microbiological procedure. Every uropathogen produced the Urease enzyme with the different activity, for example, Pseudomonas aeruginosa had the UA of 101 mmol/l, Proteus mirabilis 62 mmol/l, E. coli 54 mmol/l. The last two were the most frequent uropathogens in urine of patients with kidney stones [17,18]. In our study the most active Urease 316 mmol/l was produced by Klebsiella pneumonia.

Urine UA correlated with the chemical composition of the urine crystals and the speed of crystal formation. For instance, during the formation of phosphates the UA reached 324 mmol/l, while the formation of uric acid or oxalate crystals in the absence of acute inflammatory process was accompanied by UA of 100 mmol/l, and 58 mmol/l respectively. The highest speed of crystal formation was observed at UA values of 306mmol/l, the medium and low speed of crystal formation were 56mmol/l and 50mmol/l respectively.
The standard procedure to measure urine pH didn't detect the process of crystal formation, but pH stability test was very helpful because it registered the presence of bacteria in urine, and crystal formation due to this pathogen [12]. In the 3rd subgroup of patients with the most severe symptoms of urolithiasis pH increased from 6 to 8, and the UA numbers were highest as well: from 110 to 400 mmol/l. Both tests: pH stability and UA of urine confirmed the presence of uropathogens, that contributed to the crystal formation.

To detect the crystal formation in urine, we used two more special tests and compared the results with the urine UA. The induced crystallization urine test was very helpful, because it detected not only the presence of crystals, but also their chemical composition. If by standard procedure we could detect the crystals in the 2nd and 3d subgroups, when UA was higher than the reference numbers, with the special test we could notice the crystals in the 1st subgroup as well, when UA was within the reference numbers. It confirmed the theory that crystal formation in urine had different mechanisms besides the UA.

The AC test demonstrated the presence in the urine of patients with urolithiasis different chemical substances, both inorganic and organic, capable of crystallization in the presence of calcium ions. UA was statistically correlated with the AC test [13,14].

So, the express urine UA test is a valuable tool to record the presence of urease-producing pathogens in urine, that would contribute to the stone formation in kidney. This test has several advantages as it is the express test and does not require expensive reagents and equipment, getting it accessible to almost any clinical laboratory.

Our study had some limitations; it did not include a very large group of children with urolithiasis. In the first phase of our work, we concentrated on the studying the advantages of UA test, if it was informative, useful, correlated with other standard parameters.

In our research we included those patients who needed an urgent treatment. All 40 patients had the calculi, located in the upper third of the urinary tract, that caused renal colic, inflammation with all its consequences. Because urolithiasis has very high rate of recurrency, all patients with this disease need the metaphylaxis in the future. We are planning to continue with our research and use UA test and other lab methods for the detection of the kidney stone formation for the prevention of recurrent urolithiasis.

**Conclusion**

The urine UA is a valuable test for the diagnosis of kidney stone formation due to the presence of urease-producing bacteria in urine, that contribute to the formation of infected stones.

**Abbreviations**

AC Ability to Crystallize
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Authors’ contributions All authors contributed to the study conception and design. Material preparation and study design were performed by Assel Sagymbayeva, Natalya Merkusheva; data collection: Minira Bulegenova, Anar Musabalina; analysis interpretation of results: Assel Sagymbayeva, Natalya Merkusheva; prepared tables: Assel Sagymbayeva, Bakitzhan Abekenov; prepared figures 1-4: Assel Sagymbayeva, Abay Kussainov. The first draft of the manuscript was written by Assel Sagymbayeva and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Ethical approval This study was approved by the Ethics Committee of Asfendiyarov Kazakh National Medical University (No:2021/11-02).

Consent for publication Written informed consent was obtained from at least one guardian of each child prior to their participation in the study.

Conflict of interest The authors declare that there is no conflict of interest.

References


Figure 1

Regression line characterizing the dependence of ACon UA
Figure 2
The Correlation between UA and the type of crystals

Figure 3
The Correlation between UA and Crystal formation speed in urine
Figure 4

UA regarding to the urinary tract infections

Characteristics of the pathogen spectrum of urinary tract infections
- No
- Escherichia coli
- Enterococcus faecalis
- Staphylococcus haemolyticus
- Proteus mirabilis
- Klebsiella pneumonia
- Pseudomonas aeruginosa
- Combinations of two or more micro-organisms